

Claims 23, 27, 38 and 42 have been amended for clarity. Support is found at page 13, lines 25-26.

Claim 38 is also amended to specify the type of mutation. Support is found throughout the specification, for example, at page 7, second full paragraph.

Prior to the filing of the CPA on April 10, 2000, Claims 23-39 were subject to various rejections or objection. The Examiner is requested to consider the following remarks upon examination of the current application.

Rejection under 35 U.S.C. § 102

Claims 23, 27, 28 and 38 were previously rejected under 35 U.S.C. § 102(b) as being anticipated by Saier et al., *J. Bacteriol.* 113(1):512-514 (1973) (Saier). Applicants respectfully traverse.

For a rejection under 35 U.S.C. 102(b) to be proper, each element of the rejected claim must be disclosed in a single prior art reference (MPEP § 2131). Saier does not disclose or suggest a mutant host cell having a specific growth rate of at least 0.4 h^{-1} , nor selection of such a cell.

The claims in question are directed to a mutant host cell (Claim 23), a method for increasing PEP availability into a biosynthetic or metabolic pathway of a host cell (Claims 27 and 28), and a method for obtaining a Pts-/glucose+, galactose permease-requiring mutant cell (Claim 38). The host cell is Pts-/glu+, requires galactose permease activity to transport glucose, and has a specific growth rate on glucose as a sole carbon source of at least 0.4 h^{-1} . The method of Claims 27 and 28 calls for culturing a host cell mutant having these same characteristics in the presence of an appropriate carbon source. The method of Claim 38 calls for selecting a host cell which utilizes a phosphotransferase transport system, mutating the cell so that the phosphotransferase transport system is inactivated, culturing the mutant using glucose as a carbon source, and selecting cells having a specific growth rate of at least 0.4 h^{-1} .

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Saier discloses mutant *Salmonella typhimurium* strains lacking the genes coding for both enzyme I and HPr which grew on glucose as the carbon source with a doubling time of 2 hours. As the Examiner has noted, this translates to a specific growth rate of $.35 \text{ h}^{-1}$ (more precisely, 0.3465 h^{-1}). A specific growth rate of 0.35 h^{-1} is specifically excluded from the range of at least 0.4 h^{-1} . Furthermore, Saier neither discloses nor suggests that their mutant strains could grow at a rate faster than $.35 \text{ h}^{-1}$. Therefore, cells having the characteristics of the host cells of the present claims are not disclosed or suggested in Saier.

For the reasons discussed above, Claims 23, 27, 28 and 38 are not anticipated by Saier. Therefore, Applicants respectfully request that this 35 U.S.C. § 102(b) rejection be withdrawn.

Rejection under 35 U.S.C. § 103

Claims 23-38 were previously rejected under 35 U.S.C. § 103 as being obvious over Frost, USPN 5,168,056 (Frost), Holms, *Curr. Topics Cell. Reg.* 28:69-105 (1996) (Holms), Ingrahm et al., USPN 5,602,030 (Ingrahm) and Saier. Applicants respectfully traverse.

For a rejection under 35 U.S.C. § 103 to be proper, the prior art must: 1) teach each of the elements of the claims; 2) provide a suggestion or motivation to combine and/or modify the reference teachings; and 3) provide a reasonable expectation of success in obtaining the claimed invention by such modification/combination (MPEP § 2142-2143). Applicants submit that these criteria have not been met.

The elements of Claims 23, 27 and 38 are described above. Because each dependent claim has all of the elements of the claim from which it directly or indirectly depends, Claims 24-26 have all of the elements of Claim 23 and Claims 28-31 have all of the elements of Claim 27. Claims 33 to 37 now depend, directly or indirectly, from Claim 42. Claim 42 is directed to a method for enhancing production of a desired

compound in a modified host cell, which unmodified cell is capable of utilizing the Pts system for carbohydrate transport. The method of Claim 42 comprises culturing a modified host cell with an appropriate carbon source. The host cell has the characteristics of having a Pts-/glu+ phenotype, requiring galactose permease transport to transport glucose, having a specific growth rate of at least 0.4 h^{-1} on glucose as a sole carbon source, utilizing PEP as a metabolite, and comprising recombinant DNA encoding enzyme(s) catalyzing reaction(s) in the biosynthetic pathway of the desired compound.

The disclosure of Saier is discussed above. As discussed above, Saier neither discloses nor suggests a Pts-/glu+ host cell requiring galactose permease activity and having a specific growth rate of at least 0.4 h^{-1} . This deficiency is not remedied by combining any or all of the other references.

Frost discloses transforming cells with a transketolase gene, with or without genes encoding enzymes in the common aromatic pathway, to enhance production of aromatic pathway compounds. This reference neither discloses nor suggests a host cell with an altered glucose transport system.

Holms generally describes the central metabolic pathways in *E. coli* and the measurement of carbon flux through them. This reference provides no suggestion for a host cell with an altered glucose transport system.

Ingrahm discloses producing cells transformed with genes encoding a glucose-facilitated diffusion protein (GLF) and a hexokinase. This reference also neither discloses nor suggests a Pts-/glu+ host cell requiring galactose permease activity and having a specific growth rate of at least 0.4 h^{-1} .

MPEP § 2141.02 instructs that a claim must be considered as a whole. As a whole, as described above, all of the elements of the claim are not taught by the prior art. There is no disclosure or suggestion that a Pts disabled host cell could be mutated to have a specific growth rate of at least 0.4 h^{-1} , particularly one that required galactose permease activity to transport glucose. Nor is there any suggestion that a such a cell would benefit

from, or even survive, transformation with sequences encoding: transketolase, transaldolase or phosphoenolpyruvate to enhance levels thereof; mutated pyruvate kinase or other regulators of pyruvate kinase to reduce activity, or enzymes of the aromatic pathways. No such modified cells are disclosed or suggested. None of the cited references

Furthermore, MPEP § 2141.02 instructs that prior art must also be considered in its entirety, including disclosures that teach away from the claims. Ingrahm specifically and exclusively teaches increasing glucose uptake by introducing genes encoding GLF and hexokinase into a host cell. Therefore, this reference teaches away from innate glucose transport mechanisms, suggesting that a heterologous system must be introduced. Ingrahm also teaches that transformation with both enzymes is required to improve the growth rate of cells.

all elements not taught

As discussed above, taking the claims as a whole, all of the elements of the claims are not taught or suggested by any one or any combination of the references, and the formulation of combinations and modifications presented in the previous Office Actions do not derive obviously from the prior without the present disclosure as a guide. None of the references disclose or suggest a Pts-/glu+, galactose permease-requiring host cell having a specific growth rate of at least 0.4 h^{-1} . As such, the cells of Claims 23-26 are not obvious and the methods of the remaining claims are not obvious for lack of a key component. The addition of Ingrahm to Saier does not result in the described cell, because Ingrahm suggests the way to obtain Pts-/glu+ cells is to transform the cells with genes encoding heterologous enzymes such as GLF and hexokinase.

The Office Action suggests that the Holms disclosure that 66% of PEP goes to the Pts pathway leads the ordinary skilled artisan to contemplate increasing PEP by blocking its entry into the Pts pathway. However, the skilled artisan would not come to such an

idea based on the Holms disclosure because such action would block the only source of PEP production disclosed in the reference. Given the Holms disclosure, blocking the Pts pathway would only reduce PEP. Applicants submit that this reference provides no motivation to disrupt the Pts pathway.

The Office Action suggests that Frost renders increasing PEP obvious because it would ensure that sufficient levels of the precursors for the aromatic pathways are available. However, while Frost clearly includes PEP as a precursor to the common aromatic pathway and the pathway to PEP via the Pts pathway was well known, this clearly omits increasing PEP by any means, while disclosing several other manipulations to enhance aromatic production. This obvious omission teaches away from increasing PEP, contrary to the Office Action assertion.

Moreover, the methods claims require the recognition not only that a Pts-/glu+, galactose permease-requiring cell could have a specific growth rate of at least 0.4 h^{-1} , but also that such a cell could be used to increase PEP availability into a biosynthetic or metabolic pathway and that such a cell could be used to enhance production of a desired compound. These two elements are neither found nor suggested in the prior art.

no motivation to combine references

As discussed above, Holms provides a general picture of *E. coli* metabolic pathways. While it is useful for the skilled artisan to know such information, this reference lends nothing substantial to the production of the present invention.

While Frost is directed to enhancing aromatic pathway compounds, this reference teaches away from the present invention by suggesting that increasing PEP is not an important avenue to the desired effect and it provides no instruction at all regarding the Pts pathway. This reference is related to subsequent elements of the present invention (i.e., dependent claims 24, 29 and 33-37), but not to the elements that are fundamental to all embodiments of the invention.

As discussed above, Ingrahm teaches away from the present invention by suggesting that recombination with genes encoding GLF and hexokinase, such as those found in *Z. mobilis*, is required to obtain an organism having a glucose uptake pathway that is not coupled to PEP. That the idea of uncoupling glucose uptake and PEP production is present in the reference does not provide motivation to combine its teachings with the other cited references. Taken as a whole, this reference is directed to production of recombinant bacteria having heterologous genes encoding GLF and hexokinase. This does not relate to the disclosures of Frost or Saier, thus, there is no motivation found in this reference to combine teachings with these others.

As discussed above, Saier neither discloses nor suggests cells having a growth rate of at least 0.4 h^{-1} , nor any suggestion that Pts-/glu+ cells could have such a specific growth rate. Furthermore, Saier makes no suggestion of application of a Pts-/glu+ cell to methods of increasing PEP availability or enhancing production of a desired compound, nor is any effect on PEP or subsequent metabolic production discussed.

There is an enormous body of literature related to the central metabolic pathways of cells. The fact that several references could possibly be combined is not sufficient to establish obviousness (*see* MPEP § 2143.01). The motivation to combine must lie within the reference, without the benefit of the application's disclosure to guide such combination. Applicants submit that the inherent motivation to combine the cited references does not exist.

no reasonable expectation of success

Even if, *arguendo*, the cited references were combined and modified, there would still be no reasonable expectation of obtaining the claimed invention. No Pts-/glu+, galactose permease-requiring cell having a growth rate of at least 0.4 h^{-1} is disclosed or suggested. Therefore, the skilled artisan would have no reasonable expectation that such a cell could be obtained. Furthermore, no combination of cited references would lead to a

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reasonable expectation that such a cell could be used in a method to increase PEP availability or for enhancing production of a desired compound. The combination of references provide no evidence that a Pts-/glu+, galactose permease-requiring cell would have increased PEP without also having genes encoding GLF and hexokinase recombinantly incorporated therein.

For the reasons discussed above, Claims 23-38 are not obvious over the combined disclosures of Frost, Holms, Ingrahm and Saier. Therefore, Applicants respectfully request that this 35 U.S.C. § 103 rejection be withdrawn.

Applicants submit that the application is in form allowance and early notification of such is earnestly requested. The Examiner is invited to contact the undersigned at (415) 781-1989 if any issues may be resolved in that manner.

Respectfully submitted,

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APPENDIX

What is Claimed is

23. (Twice Amended) A mutant host cell comprising a metabolic pathway which uses PEP as a precursor or intermediate of metabolism, said host cell characterized by:
- (a) being phenotypically Pts-/glu+;
 - (b) requiring galactose permease activity to transport glucose; and
 - (c) having a specific growth rate on glucose as a sole carbon source of at least [about] 0.4h^{-1} .
24. A mutant host cell of Claim 23 comprising recombinant DNA coding for one or more of the enzymes selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase such that the mutant host cell expresses transketolase, transaldolase or phosphoenolpyruvate synthase at enhanced levels relative to wild-type host cells.
25. (once amended) A mutant host cell of Claim 23 further comprising mutations in the *pykA* and/or *pykF* genes in said host cell.
26. (once amended) A mutant host cell of Claim 24 further comprising mutations in the *pykA* and/or *pykF* genes in said host cell.
27. (Twice Amended) A method for increasing PEP availability into a biosynthetic or metabolic pathway of a host cell, the method comprising:
- culturing a host cell mutant characterized by:
 - having a Pts-/glu+ phenotype;
 - requiring galactose permease activity to transport glucose; and
 - having a specific growth rate on glucose as a sole carbon source of at least [about] 0.4h^{-1} ;
 - in the presence of an appropriate carbon source, wherein said host cell mutant utilizes PEP as a precursor or intermediate of metabolism.
28. A method of Claim 27 wherein the Pts- phenotype is caused by the deletion or inactivation of all or substantially all of one or more gene(s) selected from the group consisting of *ptsI*, *ptsH* and *crr*.
29. A method of Claim 27 further comprising modifying the selected host cell to introduce therein recombinant DNA coding one or more of the enzymes selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase such

that the mutant host cell expresses transketolase, transaldolase or phosphoenolpyruvate synthase at enhanced levels relative to wild-type host cells.

30. A method of Claim 27 further comprising modifying the selected host cell to reduce or eliminate pyruvate kinase activity in said host cell.

31. A method of Claim 30 wherein pyruvate kinase activity is reduced or eliminated in the host cell by introducing a mutation in DNA encoding one or more of the sequences coding for pyruvate kinase, pyruvate kinase promoter region and other regulatory sequences controlling expression of pyruvate kinase.

33. (once amended) A method of Claim 42 wherein the DNA used to transform the host cell encodes one or more enzyme(s) selected from the group consisting of DAHP synthase, DHQ synthase, DHQ dehydratase, shikimate dehydrogenase, shikimate kinase, EPSP synthase and chorismate synthase.

34. (once amended) A method of Claim 42 further comprising transforming the host cell with recombinant DNA coding one or more enzyme(s) selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase so that said enzyme is expressed at enhanced levels relative to wild-type host cells.

35. A method of Claim 33 further comprising transforming the host cell with recombinant DNA coding one or more enzyme(s) selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase so that said enzyme is expressed at enhanced levels relative to wild-type host cells.

36. (once amended) A method of Claim 42 wherein the desired compound is selected from the group consisting of tryptophan, tyrosine and phenylalanine.

37. A method of Claim 36 wherein the desired compound is tryptophan and the host cell is transformed with DNA coding one or more gene(s) selected from the group consisting of *aroG*, *aroA*, *aroC*, *aroB*, *aroL*, *aroE*, *trpE*, *trpD*, *trpC*, *trpB*, *trpA* and *tktA* or *tktB*.

38. (Twice Amended) A method for obtaining a Pts-/glucose⁺, galactose permease requiring-mutant cell, the method comprising:

- (a) selecting a host cell which utilizes a phosphotransferase transport system;
- (b) mutating the host cell whereby the phosphotransferase transport system is inactivated;
- (c) culturing the mutant host cell using glucose as a carbon source; and

(d) selecting a mutant host cell which grows on glucose at a specific growth rate of at least [about] 0.4 h^{-1} .

39. (once amended) A method of Claim 38 wherein the mutant cells are selected due to a specific growth rate on glucose of about 0.8 h^{-1} .

40. The mutant cell of Claim 23 having a specific growth rate on glucose as a sole carbon source of about 0.8 h^{-1} .

41. The mutant cell of Claim 23 wherein the Pts- phenotype is caused by the deletion or inactivation of all or substantially all of one or more gene(s) selected from the group consisting of ptsI, ptsH and crr.

42. A method for enhancing production of a desired compound in a modified host cell, said host cell in its unmodified form being capable of utilizing a phosphotransferase transport system for carbohydrate transport, the method comprising,
(a) culturing a modified host cell with an appropriate carbon source, said modified host cell characterized by having:
 (i) a Pts-/glu+ phenotype;
 (ii) requiring galactose permease activity to transport glucose;
 (iii) [having] a specific growth rate on glucose as a sole carbon source of at least [about] 0.4 h^{-1} ; and
 (iv) utilizing PEP as a precursor or intermediate of metabolism,
said modified host cell further comprising recombinant DNA encoding one or more enzyme(s) catalyzing reactions in the pathway of biosynthetic production of said desired compound in said modified host cell; and
(b) optionally recovering said compound.

43. The mutant cell of Claim 42 having a specific growth rate on glucose as a sole carbon source of about 0.8 h^{-1} .

44. The mutant cell of Claim 42 wherein the Pts- phenotype is caused by the deletion or inactivation of all or substantially all of one or more gene(s) selected from the group consisting of ptsI, ptsH and crr.

45. The method of Claim 38 wherein mutating the host cell is by inactivating the phosphotransferase transport system.

46. The method of Claim 45 wherein said inactivating is by deleting part or all of gene(s) selected from the group consisting of ptsI, ptsH and crr.